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INSTITUTE REPORT NO. 224

PRIMARY DERMAL IRRITATION POTENTIAL OF TRIMETHYLOLETHANE TRINITRATE (TMETN) IN RABBITS

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Primary Dermal Irritation Potential of Trimethylolethane Trinitrate (TMETN), (Toxicology Series 114)--Morgan and Korte

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The primary dermal irritation potential of trimethylolethane trinitrate (TMETN) was determined in male and female New Zealand White rabbits using a modified Draize method. The test compound was non-irritating. Very slight erythema was observed in 2 rabbits by 1/2 hour after dosing and in 4 rabbits at 24 hours after dosing. All rabbits had returned to normal by 48 hours after dosing. Neither edema nor any other recognizable skin reaction was detected at any time during the 14-day observation period.

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ABSTRACT

The primary dermal irritation potential of trimethylolethane trinitrate (TMETN) was determined in male and female New Zealand White rabbits using a modified Draize method. The test compound was non-irritating. Very slight erythema was observed in 2 rabbits by 1/2 hour after dosing and in 4 rabbits at 24 hours after dosing. All rabbits had returned to normal by 48 hours after dosing. Neither edema nor any other recognizable skin reaction was detected at any time during the 14 day observation period.

Key words: Primary Dermal Irritation, Trimethylolethane Trinitrate, TMETN, Mammalian Toxicology, Rabbit

PREFACE

TYPE REPORT: Primary Dermal Irritation GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR: US Army Medical Research and Development Command
US Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, Maryland 21701-5010
Project Officer: Gunda Reddy, PhD

WORK UNIT: 3E162720A835
Toxicity Testing of Propellants
WU 180; APC TL09

GLP STUDY NUMBER: 84043

STUDY DIRECTOR: MAJ Don W. Korte Jr., PhD, MSC

PRINCIPAL INVESTIGATOR: CPT Earl W. Morgan, DVM, VC
Diplomate of American College of
Veterinary Preventive Medicine

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, retired SOPs, raw data, analytical, stability, and purity data of the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Trimethylolethane Trinitrate

INCLUSIVE STUDY DATES: 24 October - 13 November 1984

OBJECTIVE: The objective of this study was to determine the primary dermal irritation potential of Trimethylolethane Trinitrate in male and female New Zealand White rabbits.

ACKNOWLEDGMENT

SGT Steven K. Sano, BS and Yvonne C. Johnson, BS, assisted in the research; SP4 James J. Fisher, SP4 Scott L. Schwebe, and Charlotte Speckman provided care for the animals; and Callie B. Crosby, MA, and Brenda V. Goce, provided secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 84043, was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr. 7 MAY 85

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REPLY TO ATTENTION OF

SGRD-ULZ-QA

14 January 1986

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

1. I hereby certify that in relation to LAIR GLP Study 84043 the following inspections were made:

6 November 1984

2. The report and raw data for this study were audited on 10 October 1985.

3. Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the 21 January 1985 report to Management and the Study Director.

GARY L. DUTCHER
SSG, USA
Quality Assurance Unit

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Primary Dermal Irritation Potential of Trimethylolethane Trinitrate in Male and Female Rabbits--Morgan et al

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in munition formulations. A "health effects" review conducted for the US Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) identified numerous gaps in the toxicology database of these compounds (1). Consequently, USAMBRDL has tasked the Toxicology Branch, LAIR, to conduct an initial health effects evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP. This initial evaluation includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, acute dermal toxicity tests in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs. This report contains the results of a study to assess the primary dermal irritation potential of TMETN, in rabbits.

Objective of Study

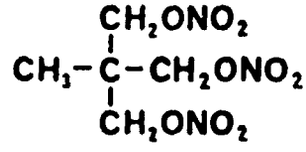
The objective of this study was to determine the primary dermal irritation potential of TMETN in male and female New Zealand White rabbits.

MATERIALS

Test Substance

Chemical name: Trimethylolethane Trinitrate

Chemical structure:



Molecular formula: $C_5H_9N_3O_9$

Other test substance information is presented in Appendix A.

Animal Data

Four male and four female New Zealand White rabbits (Elkhorn Rabbitry, Watsonville, CA), identified individually with ear tattoos, numbered 84F596 to 84F597, 84F599 to 84F603, inclusive, and 84F611 were assigned to the study. The animal weights on dosing day (30 Oct) ranged from 3.0 to 3.5 kg. Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel, screen-bottomed, battery-type cages with automatically flushing dumptanks. The diet consisted of 150 g per day of Certified Purina Chow Diet 5322 (Ralston Purina Company, St Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained at 20.0 to 22.2°C with a relative humidity range of 48 to 66 percent with short spikes up to 82 percent associated with room cleaning. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Study animals were acclimated for 6 days to the study room following a 14-day quarantine by the Animal Resources Group. During this period they were observed daily for signs of illness. They were treated once prophylactically for ear mites with Canex® and mineral oil in the ears.

Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP-OP-STX-34 (3).

The backs of 8 rabbits were close-clipped 24 hours before the actual dosing. The clipped area was divided into 4 quadrants designated I-V (4, 5). Sites I and IV were sham patches. Sites II and III were test compound sites. Since the TMETN is a liquid, a standard dose of 0.5 ml of the test compound was placed on 1-inch (2.5 cm) square gauze patches which were taped to Sites II and III. Blenderm®, (Medical Products Division of 3M, Saint Paul, MN), a semiimpervious, hypoallergenic surgical tape, was used to hold the patches in place. Vet Wrap® (Animal Care Products Division of 3M, Saint Paul, MN) was then wrapped securely around the animal. The test compound was left in contact with the skin for 4 hours. At the end of the exposure period the wrapping and patches were removed, and the areas were scored 1/2 hour later.

Observations

The grading and scoring for dermal reactions were performed according to Table 1. Observations were made daily from 30 October to 13 November 1984. Scoring and grading were performed at 1/2, 24, 48, and 72 hours after removal of the patch.

TABLE 1
EVALUATION OF SKIN REACTIONS

Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate-to-severe erythema	3
Severe erythema (beet redness to slight eschar formation (injurious in depth)	4
Possible total erythema score:	4*
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (edges raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Possible total edema score	4*
Possible total score for primary irritation	8

*Any skin reaction more serious than severe edema, vesiculation, ulceration, or necrosis places the chemical in Category V.

Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

Rabbit number 84F601 had the test compound and the sham patch sites inadvertently reversed. All other aspects of this study were conducted in accordance with all applicable SOPs and addenda.

Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound were retained in the LAIR Archives.

RESULTS

Animals were scored for erythema and edema at each patch site. Five rabbits exhibited very slight erythema (score 1) at test compound sites. Rabbits 84F597 and 84F602 were observed to have very slight erythema at 1/2 hour after dosing. Rabbits 84F596, 84F597, 84F600, and 84F611 exhibited very slight erythema 24 hours after dosing. All rabbits had returned to normal by 48 hours after dosing. Neither edema nor any other recognizable skin reaction was detected at any time during the 14-day observation period. The sham patch sites were normal throughout the study. Results of scoring the dermal irritation potential in each rabbit were tabulated (Appendix D).

DISCUSSION

TMETN produced very slight erythema at the test patch sites in five of eight rabbits after a 4-hour dermal patch test. Neither edema nor any other recognizable skin reaction was detected at any time during the 14-day observation period.

The primary irritation index adapted from McCreesh and Steinberg (5) was used as a basis for categorization. Non-irritating compounds (Category I) have peak net mean scores of 0.0-0.5. Mild irritants (Category II) have peak net mean scores from 0.51 to 2.0. Category III Compounds are moderately irritating with indices between 2.1 and 5.0. Chemicals are considered severe irritants (Category IV) if they have indices between 5.1 and 7.9 and they produce necrosis, vesiculation, ulceration, and/or eschars. Compounds which are impossible to classify because of staining or masking of effects due to physical properties are placed in Category V. The peak net mean score from the test compound was 0.5. Therefore, TMETN was classified as a non-irritating chemical (Category I).

Jones et al (6) studied the toxicity of Propylene Glycol 1, 2-dinitrate (PGDN) a nitrate ester structurally similar to TMETN. Their studies showed that PGDN was absorbed percutaneously but produced no primary dermal irritation at either 24 or 72 hours after exposure to vapor. The results of this study with TMETN are consistent with the non-irritancy reported for PGDN.

CONCLUSION

The test compound, TMETN, is a non-irritant under conditions of this assay.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No ADA 127846.

2. Environmental Protection Agency. Office of Pesticide and Toxic Substances, Office of Toxic Substances (TS-792). Primary Dermal Irritation. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.

3. Primary Dermal Irritation Study. LAIR Standard Operating Procedure OP-STX-34, Letterman Army Institute of Research, Presidio of San Francisco, CA. 1 August 1984.

4. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944; 85:377-390.

5. McCreesh AH, Steinberg M. Skin irritation testing in animals. In: Marzulli FN, Maibach HI, eds. Dermato-toxicology and pharmacology (Advances in modern toxicology, vol 4). Washington: Hemisphere Publishing Corp, 1977: 193-210.

6. Jones RA, Strickland JA, Siegel J. Toxicity of Propylene Glycol 1,2-Dinitrate in experimental animals. Tox Appl Pharmacol 1972; 22: 128-137.

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Appendices

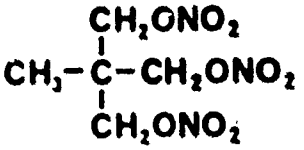
CHEMICAL DATA

Chemical Name: 1,3-Propanediol, 2-methyl-2 [(nitrooxy)methyl]-dinitrate (ester)

Other Listed Names: 1,3-Propanediol-2-(hydroxymethyl)-2-methyl-, trinitrate; 1,1,1-Trimethylolethane trinitrate (TMETN), Metriol trinitrate (MTN); Nitropentaglycerin

CAS: 3032-55-1

Structural formula:



Molecular formula: $C_5H_9N_3O_9$

Molecular weight: 255.15

Physical state: Light brown oil

Melting Point: -3°*†

Compound Density: 1.47 g/cm *†

Lot No: 53-84A

Source: Naval Ordance Station
Indian Head, MD

Chemical Analysis

Instrumentation:
Ultra-violet (UV) spectra were obtained using a Hitachi 110-A Spectrophotometer (Hitachi Instruments, Inc., Mountain View, CA), infra-red spectra (IR) were obtained with a Perkin-Elmer Model 457 Infra-red Spectrophotometer (Perkin-Elmer, Norwalk, CT) and nuclear magnetic resonance (NMR) spectra were recorded on a Varian FT-80 NMR (Varian, Palo Alto, CA) using tetramethylsilane as an internal standard. Chromatographic analysis was performed using a 1090B HPLC with diode array detector (Hewlett-Packard, Santa Clara, CA) and a Brownlee RP-18 Spheri-5 Column, 4.6 x 250 mm (Brownlee Labs, Inc., Santa Clara, CA). The following conditions were employed for the HPLC assay: solvent system, 70% methanol, 30% water; flow rate 0.9 ml/min; detector wavelength, 215 nm; oven temperature, 50°C.

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* Holleman JW, Ross RH, Carroll JW. Problem definition study on health health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: U.S. Army Medical Bioengineering Research and Development Laboratory, 1983; DTIC No. ADA 127846, p17.

† Lindner V. Properties of explosive aliphatic nitrate esters. Table 5. In: Grayson M., exe. ed. Kirk-Othmer encyclopedia of chemical technology. Volume 9. 3rd ed. New York: John Wiley and Sons, Inc., 1980:573.

Results:

UV Spectrum: For UV analysis TMETN was dissolved in acetonitrile. UV absorbance begins at approximately 240 nm and increases with decreasing wavelength.* No absorption peak was observed. IR (KBr windows): 2900, 1645 (asymmetric stretch of NO group), 1470, 1375, 1280 (symmetric stretch of NO_2 group), 990, 860, and 755 cm .† ^1H NMR ($CDCl_3$, 80MHZ): 61.22 (S, 3H, CH_3), 4.44 (S, 6H, $-CH_2-$).† TMETN subjected to HPLC analysis eluted as two peaks with retention times of 8 and 12.5 min.§ Based on integration of peak areas the first peak represented 98% of the sample. The second peak was not identified. No decomposition of TMETN was detected by HPLC after storage of TMETN (neat or in ethanol) for a period of nine weeks.‖

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*Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010, p 51. Letterman Army Institute of Research, Presidio of San Francisco, CA.

†Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p 67. Letterman Army Institute of Research, Presidio of San Francisco, CA.

‡Ibid., p 68.

§Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010, p 72-75. Letterman Army Institute of Research, Presidio of San Francisco, CA.

‖Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p 34. Letterman Army Institute of Research, Presidio of San Francisco, CA.

ANIMAL DATA

Species: Oryctolagus cuniculus

Strain: New Zealand White (albino)

Source: Elkhorn Rabbitry
5265 Starr Way
Watsonville, CA 95076

Sex: Male and Female

Age: Young Adults

Animals in each group: 4 males and 4 females

Condition of animals at start of study: Normal

Body weight range at dosing: 3.0 - 3.5 Kg

Identification procedures: Ear tag, tag numbers 84F596-84F597; 84F599-84F603, and 84F611 inclusive.

Pretest conditioning:

1. Quarantine from 11 October - 29 October 1984

2. Animal were close-clipped and examined 24 hours before dosing.

Justification: Laboratory rabbits are a proven sensitive animal model for dermal irritation.

HISTORICAL LISTING OF STUDY EVENTS

Date	Event
11 Oct 84	Rabbits arrived LAIR.
12 Oct 84	They were tattooed, weighed, examined for illness, and placed under a two week quarantine.
11-23 Oct 84	Animals were checked daily by Animal Resources Group (ARG) personnel.
24 Oct 84	All rabbits were treated with Canex® and mineral oil in their ears. Rabbits were removed from quarantine after being certified healthy by ARG Staff Veterinarian. The animals were weighed.
25-29 Oct 84	Animals were checked daily.
29 Oct 84	Animals were close clipped and areas marked.
30 Oct 84	Animals were weighed. Test substance was applied for four hours. Patches were removed and sites scored within 60 minutes.
31 Oct-13 Nov 84	Animals were observed daily.
31 Oct- 2 Nov 84	Areas were scored at 24, 48, and 72 hours after exposure.
5 Nov 84	Reclipped animals.
6 Nov 84	Animals were weighed.
13 Nov 84	Animals were weighed and sacrificed.

SUMMARY OF PRIMARY IRRITATION TEST DATA

Animal No.	30-60 Min Test	30-60 Min Sham	30-60 Min Vehicle	24 h Test	24 h Sham	24 h Vehicle	48 h Test	48 h Sham	48 h Vehicle	72 h Test	72 h Sham	72 h Vehicle
84F596	0	0	0	1	0	0	0	0	0	0	0	0
597	1	0	0	1	0	0	0	0	0	0	0	0
599	0	0	0	0	0	0	0	0	0	0	0	0
611	0	0	0	1	0	0	0	0	0	0	0	0
600	0	0	0	1	0	0	0	0	0	0	0	0
601	0	0	0	0	0	0	0	0	0	0	0	0
602	1	0	0	0	0	0	0	0	0	0	0	0
603	0	0	0	0	0	0	0	0	0	0	0	0
Mean*	.25	0	0	.5	0	0	0	0	0	0	0	0
Net Mean Score†	.25	0	0	.5	0	0	0	0	0	0	0	0

*Peak net mean score for test compound .5; Primary Skin Irritation Category I

†Test Mean Value - (greater of sham or vehicle mean value)

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